Desiccant Dusts Synergize the Effect of *Beauveria bassiana* (Hyphomycetes: Moniliales) on Stored-Grain Beetles

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ABSTRACT   Diatomaceous earth (DE) is a desiccant insecticide and most efficacious in low humidity. It acts on insect cuticle by absorbing lipids, and perhaps by cuticular abrasion. *Beauveria bassiana* (Balsamo) Vuillemin, an entomopathogenic fungus, is most efficacious in high humidity and has a complex interaction with cuticular lipids. Interaction between these materials may enhance insect control performance. Assays with stored-grain beetles were conducted with *B. bassiana* at rates of 11, 33, 100, and 300 mg of conidia per kilogram of grain with and without single rates of DE that killed 10% or less of the target beetles. The assays revealed synergism in effects on adult *Rhyzopertha dominica* (F.) and *Oryzaephilus surinamensis* (L.) at all doses. There was statistically significant synergism for adult *Cryptolestes ferrugineus* (Stephens) and larval *R. dominica* but at only one *B. bassiana* rate for each target. Both amorphous silicon dioxide, a sorptive dust, and diamond dust, an abrasive, showed synergistic interaction with *B. bassiana* on adult *R. dominica*. These results may provide a basis for a least-toxic approach to control of stored-product beetles and for efficacy-enhancing formulation of entomopathogenic fungi.

KEY WORDS   *Beauveria bassiana*, synergism, mycopathogen, stored grain, diatomaceous earth, Coleoptera

DIATOMACEOUS EARTH (DE) is a nontoxic and stable alternative to traditional insecticides, but even modest amounts can cause unacceptable adverse effects on the physical properties of grain as well as dust (silica) inhalation problems when used in enclosed food processing areas (Golob 1997, Korunic 1998). Because it acts as a desiccant, DE is most active at low humidity. *Beauveria bassiana* (Balsamo) Vuillemin is a fungus that infects and kills a broad range of insects. It is registered by the U.S. Environmental Protection Agency (EPA) for a wide range of insect control uses, but is of unproven efficacy at practical dose rates for stored grain pests. As is the case with most entomopathogenic fungi, its performance is enhanced by high humidity (Moore et al. 2000). Accordingly, the two materials are complementary in their moisture optima.

Both DE and *B. bassiana* act through the insect integument but in different ways that may result in a significant interaction. Desiccant dusts diminish the water retention ability of insects by removal of cuticular lipids (Quarles and Winn 1996), although Wigglesworth (1944, 1947) demonstrated that desiccation of insects treated with alumina was due to abrasion. Thus, DE appears to dehydrate by both lipid adsorption and abrasion (Ebeling 1971). *Beauveria bassiana* has complex and incompletely understood interactions with cuticular lipids. Certain cuticular lipids, including short chain fatty acids (Smith and Grula 1982) and certain aldehydes (Sosa-Gomez et al. 1997), are inhibitory to entomogenous fungi. However, long-chain hydrocarbons may play an important role in initial attachment of *B. bassiana* spores to cuticle given that such dry phialoconidia attach in a nonspecific manner through hydrophobic interaction (Boucias et al. 1988). *Beauveria bassiana* can use insect epicuticular hydrocarbons as a sole carbon source (Napolitano and Juarez 1997), and its growth is enhanced by the presence of C18 fatty acids (Bidochka and Khachatourians 1992). Clearly the lipid components of insect cuticle include both antifungal factors and factors that can promote successful invasion of the host.

The goal of the work presented here was to determine whether there is a synergistic interaction between desiccant dusts and entomopathogenic fungi that can be exploited to improve formulations and enhance their performance as insect control agents, thereby reducing use rates. Additionally, the information gleaned might provide a basis for studies on cuticular factors that affect spore attachment and germination.

Materials and Methods

A single commercially produced lot of unformulated conidiospores of *B. bassiana* isolate GHA (Mycotech, Butte, MT) was used in all experiments. It

contained 6.3×10^{10} conidia per gram and had a germination rate of >90% as assessed after 16 h of incubation at 26°C on Sabouraud dextrose agar (SDA). The DE preparation was a commercial insecticide formulated with 10% silica gel (Protect-It, Hedley Technologies, Blaine, WA). It contained a wide variety of particle sizes and shapes with elongate particles to 150 μm and rounded particles from 1 to 50 μm diameter. Amorphous silica (Sigma, St. Louis, MO) of 0.5–10 μm diameter and diamond powder (Aldrich, Milwaukee, WI) of ≈1 μm diameter were used as absorbent and abrasive desiccant dusts, respectively (Ebeling 1971).

Assays were run with cotton-plugged 13 by 100-mm test tubes with 5 g of hard red winter wheat of 12–13% moisture and 10 beetles per tube, four tubes per treatment per assay. Each experiment was repeated three times. Repetitions of the assays were done with separate generations of insects from laboratory colonies. All of the test insects were from colonies maintained at the Grain Marketing and Production Research Center, Manhattan, KS. Adult lesser grain borers, *Rhyzopertha dominica* (F.), sawtoothed grain beetles, *Oryzaephilus surinamensis* (L.), and rusty grain beetles, *Cryptolestes ferrugineus* (Stephens) were of mixed age and sex. Survival among adult beetles was scored 8 d after treatment. For treatment of larval *R. dominica*, 10 eggs were placed in each treatment tube, and their survival to adulthood was scored after 8 wk. The treatments were mixed into whole grain for lesser grain borers and crimped grain for sawtoothed and rusty grain beetles. *Beauveria bassiana* was applied at 11, 33, 100, and 300 mg of conidia per kilogram of grain (parts/million). The DE concentration was 200 mg of dust per kilogram of grain for adult lesser grain borers and 50 mg/kg for all others. The two DE dose rates were chosen to cause <10% mortality, to be within labeled use rates, and to be at rates that minimize loss of grain bulk density if applied to wheat (Korunic et al. 1998).

In an effort to further determine the relative contributions of desiccation and abrasion to *B. bassiana*–dust interaction, adult lesser grain borers and the above *B. bassiana* doses were assayed with two other materials. Amorphous silica and diamond powder were used at 1,200 and 1,000 mg/kg of grain, respectively. There were four replicates of each treatment in each experiment, and each experiment was repeated three times using beetles of a single generation. The amorphous silica and diamond dust assays were carried out with beetles of a single generation. Repetitions of other assays were done with separate generations. All incubation was at 26 ± 1°C and 75 ± 1% RH over saturated NaCl solution.

Log-probit regressions were calculated with an U.S. Environmental Protection Agency probit program (EPA 1989). Expected mortality for each concentration of *B. bassiana* with DE was calculated with the formula $P_e = P_1 + (1 - P_1)(P_2)$, where P_e = expected mortality, P_1 = mortality from *B. bassiana*, and P_2 = mortality from DE. Significant synergism (1 df, α = 0.05) was detected by a χ^2 value >3.84 from the equation $\chi^2 = (L_o - L_e)/L_e + (D_o - D_e)/D_e$, where L_o = observed number of living insects, L_e = expected number of living insects, D_o = observed number of dead insects, and D_e = expected number of dead insects (Poch et al. 1995). For lesser grain borer immatures, the only test insects with control mortality, the data were adjusted with Abbott's formula (Abbott 1925). To test for homogeneity among the three replicates of each assay, untransformed mortality data were subjected to analysis of variance, and trial means were compared with Student–Newman–Keuls test (SAS Institute 1999).

To assess relative spore attachment, conidia were stained for 1 h at room temperature in 1 mg/ml fluorescein isothiocyanate in 0.05 M carbonate-bicarbonate buffer with 0.1% Tween 80. The conidia were then washed with the buffer, air-dried, and viability in excess of 90% was confirmed. Approximately 200 adult lesser grain borers were placed in wheat with 0.2 mg stained *B. bassiana* conidia with and without 0.2 mg DE/g of grain. After 48 h of incubation, the beetles were washed three times by vortexing for 15 s in excess 0.05% Silwet L-77 (Loveland Industries, Greeley, CO). The ventral abdomens of 80 beetles from each treatment were scored for attached spores in single microscope fields at 400× under fluorescent illumination with an excitation wavelength of 460–490 nm, chromatic mirror at 505 nm, and barrier filter of 515–550 nm.

The long-term DE effect on *B. bassiana* spore viability was assessed by enclosing 0.5 g of pure spores and 0.5 g of spores with an equal amount of DE in small glass vials. Three replicates of each treatment were incubated at 26 and 30°C for 75 d. The initial and final germination rates were assessed after 16 h of incubation on SDA at 26°C.

Results and Discussion

Adult Beetles. When beetles were exposed to the various test rates of Protect-It without *B. bassiana*, the average mortalities were 2.5% for lesser grain borer adults, 9.9% for lesser grain borer immatures, 6.7% for rusty grain beetles, and 1.7% for sawtoothed grain beetles (Table 1).

There was significant synergism between DE and *B. bassiana* for all of the insects tested with at least one of the fungus doses (Table 1). Chi-square analyses of predicted and observed mortalities showed synergism between DE and *B. bassiana* at all tested fungus concentrations for lesser grain borers and sawtoothed grain beetles adults (α = 0.05). When assayed against adult lesser grain borers, the median lethal concentration of *B. bassiana* was 102 (95% CL = 86–121) mg/kg of wheat without DE and 26 (95% CL = 20–32) mg/kg with the inclusion of 200 mg of DE/kg of wheat (Fig. 1; Table 2). Similarly, for sawtoothed grain beetles, the median lethal *B. bassiana* concentration was 118 (95% CL = 94–155) mg/kg of wheat without DE and 48 (95% CL = 38–60) mg/kg with the inclusion of 50 mg of DE/kg of wheat. There was synergism between the two materials for rusty grain beetle adults

Table 1. Mortality response of stored-grain beetles to *Beauveria bassiana* on wheat with and without diatomaceous earth (significant chi-square for interaction = 3.84)

B. bassiana rate (mg conidia/kg grain)	% mortality			χ^2
	B. bassiana without DE $(\pm SD)^a$	Observed with DE $(\pm SD)$ + *B. bassiana*	Expected with DE (if additive)	
Rhyzopertha dominica adults				
0		2.5 (2.56)		
11	5.1 (0.13)	29.2 (3.82)	7.5	67.8
33	15.2 (6.41)	60.8 (7.64)	17.8	132.2
100	46.2 (7.12)	78.2 (2.75)	50.5	37.6
300	84.2 (10.10)	95.8 (1.44)	83.6	9.7
Rhyzopertha dominica immatures				
0		9.9 (13.60)		
11	22.5 (5.63)	25.2 (10.23)	30.2	1.2
33	29.7 (7.15)	30.6 (5.63)	36.7	1.6
100	38.7 (6.80)	45.0 (20.46)	44.8	—
300	69.4 (10.92)	85.6 (3.12)	67.0	8.7
Cryptolestes ferrugineus adults				
0		6.7 (3.76)		
11	23.5 (9.80)	24.2 (15.07)	28.6	0.9
33	28.5 (9.93)	48.8 (8.89)	33.3	10.8
100	45.0 (8.66)	54.2 (7.22)	48.7	1.2
300	64.6 (13.67)	73.8 (19.28)	67.0	—
Oryzaephilus surinamensis adults				
0		1.7 (2.89)		
11	11.8 (3.86)	29.5 (7.50)	13.3	22.8
33	17.7 (2.75)	42.5 (2.50)	19.1	35.4
100	47.7 (11.8)	64.6 (12.36)	48.6	10.3
300	71.5 (8.17)	84.3 (10.97)	72.0	7.5

—, Values not calculated because of variation between assays.
^{a} Standard deviation of three trial means.

only at a *B. bassiana* concentration of 33 mg/kg of wheat. The latter result may be artifactual because the mortality with DE plus *B. bassiana* was above the log-probit regression line and the mortality with *B. bassiana* alone was below the regression line. However, the difference in mortality between the two
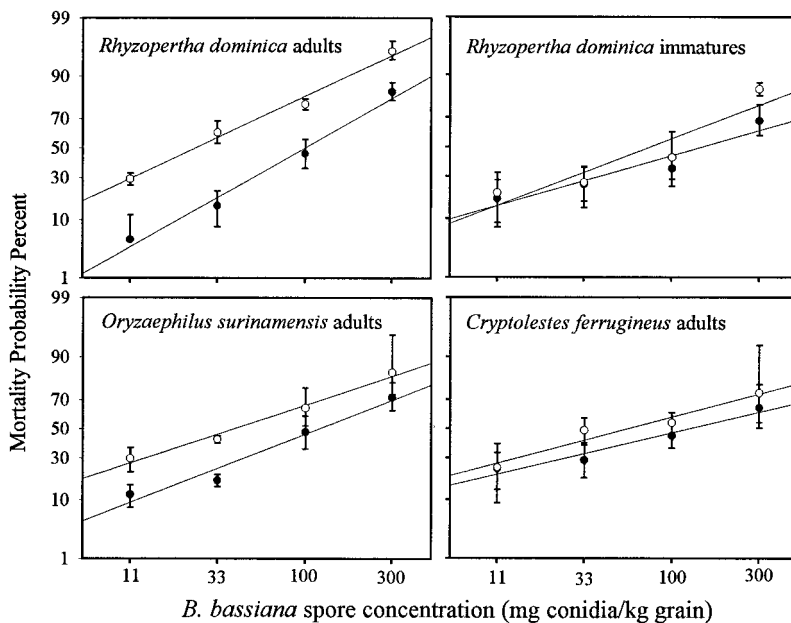

Fig. 1. Log dose-probit mortality lines for *Beauveria bassiana* treatment of stored-grain beetles on wheat with (○) or without (●) the inclusion of low doses of diatomaceous earth. Points are mean mortalities ± SD from three trials.

Table 2. Probit analyses of *Beauveria bassiana* dose-mortality data of stored-product beetles treated with or without diatomaceous earth (DE) ($n = 120$ for each dose-insect combination)

Beetle	DE	Slope (±SE)	LC$_{50}$ (95% CL)	LC$_{95}$ (95% CL)	χ^2
Rhyzopertha dominica adults	−	1.91 (0.15)	102 (86–121)	737 (526–1,157)	2.79
	+	1.51 (0.14)	26 (20–32)	315 (216–522)	1.96
Rhyzopertha dominica immatures	−	1.00 (0.11)	149 (96–243)	6,543 (2,269–50,273)	4.75
	+	1.44 (0.49)	92[a]	1,280[a]	12.53*
Cryptolestes ferrugineus adults	−	0.80 (0.11)	119 (83–187)	13,447 (4,002–112,837)	1.86
	+	0.91 (0.11)	59 (44–81)	3,847 (1,601–16,046)	4.69
Oryzaephilus surinamensis adults	−	1.31 (.013)	118 (94–155)	2,137 (1,186–5,029)	3.61
	+	1.26 (0.12)	48 (38–60)	984 (588–2,066)	0.10

*, $P < 0.05$.

[a] 95% CL not calculable because of poor fit.

treatments was consistent among the three repetitions of the assay (19, 20, and 22%). A chi-square could not be calculated for fungus–DE interaction on rusty grain beetles at a concentration of 300 mg/kg because of variation among the assays with DE included ($F = 5.5$, df = 2, $P = 0.027$).

Sawtoothed grain beetles and rusty grain beetles are similar in physical structure and feeding habits. Although the slope of the dose-mortality line is lower for rusty grain beetles, their mortality responses to DE and their LC$_{50}$s for *B. bassiana* are similar (Table 2). It seems surprising that there is a significant *B. bassiana*–DE synergism for sawtoothed grain beetles but no apparent interaction for rusty grain beetles. An explanation is elusive, but the differences were small and may not be biologically meaningful.

Lesser Grain Borer Immatures. Lesser grain borer immatures responded differently from the adult beetles. Log dose-probit mortality regressions for the adult beetles all had correlation coefficients of at least 0.95. For lesser grain borer immatures, the r^2 values were 0.909 for *B. bassiana* alone and 0.864 for the combination. The lower r^2 values reflect an increase in mortality at the highest test dose, especially for the combination. Only at the highest dose was the mortality for the combination significantly higher than the predicted additive response. This is in contrast to the responses of the adult beetles, all of which showed the greatest interaction at the lower dose of 33 mg *B. bassiana* per kilogram of grain. Given the deviations of the responses to the highest rate of fungus from the values predicted by regression, the calculated synergism at the high dose may be a statistical artifact. A chi-square could not be calculated for fungus–DE interaction on lesser grain borer immatures at a *B. bassiana* concentration of 100 mg/kg because of variation among the assays with DE included ($F = 13.6$, df = 2, $P = 0.018$).

Mode of Fungus–Desiccant Interactions. There are several plausible explanations for synergism between *B. bassiana* and diatomaceous earth. Silicaceous dusts, such as DE, remove cuticular lipids (Ebeling 1971). The first step in the infection process for entomopathogenic fungi is attachment to the lipid-covered epicuticle. The lipid composition of an insect's cuticle plays an important role in determining whether entomopathogenic fungi can attach and penetrate. Insect cuticular lipids interact with both entomogenous fungi and silicaceous dusts in highly complex and poorly understood ways. For example, Lecuona et al. (1997) reported that a pentane extract from the cuticle of the scarab *Melolontha melolontha* L., inhibited germination and growth of a nonpathogenic strain of *B. bassiana*, but did not inhibit a pathogenic strain of *Beauveria brogniartii* (Saccardo) Petch. In the same study, pentane extracted alkanes and alkenes from *Ostrinia nubilalis* (Hübner) cuticle inhibited growth, but not germination of *B. bassiana* and *B. brogniartii*. Methanol extracts from *O. nubilalis* cuticle inhibited germination, but not growth of both fungi. Koidsumi (1957) reported that removal of cuticular lipids from larval *Bombyx mori* L. and *Chilo simplex* Butler with alumina dust or carbon tetrachloride increased infection by the both the entomopathogen *B. bassiana* and the opportunist *Aspergillus flavus* Link. Koidsumi (1957) also reported that cuticular extracts thought to contain medium length saturated fatty acids were fungistatic or fungicidal according to concentration. Fatty acids of 5–12 carbon length have been implicated in fungistatic effects on *B. bassiana* and other fungi (Smith and Grula 1981, Saito and Aoki 1983). The fungistasis is concentration-dependent and can be overcome by the presence of certain nutrients (Smith and Grula 1982). On the migratory grasshopper, *Melanoplus saguinipes* (F.), Bidochka and Khachatourians (1992) did not find fatty acids that were strongly inhibitory to *B. bassiana,* but neither were those present good carbon sources for germination. Conidia germinated faster on hexane-washed than on untreated cuticle, suggesting that lipid removal improved access to more nutritious substances below. Diatomaceous earth may act by removing fungicidal and fungistatic lipids and by improving spore access to nutrients.

Abrasion of the cuticle is an alternative explanation for the observed synergism. Hunt et al. (1984) attributed increased germination of *B. bassiana* conidia on sonicated *Dendroctonus ponderosae* Hopkins adults to release of nutrients. If there is significant cuticle abrasion by DE or if removal of surface lipids makes underlying nutrients more available, then nutrient enhanced germination could contribute to increased infection of stored-grain beetles.

Table 3. Mortality response of adult *Rhyzopertha dominica* to *Beauveria bassiana* on wheat with and without desiccant dusts (significant chi-square for interaction = 3.84)

B. bassiana rate in mg/kg grain	% mortality						
	Without desiccant (±SD)[a]	Silica		χ^2	Diamond dust		χ^2
		Observed	Expected		Observed	Expected	
0	0	4.2 (5.20)			10.0 (15.21)		
11	10.0 (2.50)	20.0 (9.01)	13.8	3.29	15.0 (2.50)	19.0	1.04
33	12.5 (4.33)	45.0 (10.0)	16.1	61.47	22.5 (9.01)	21.2	0.09
100	39.2 (11.55)	87.5 (2.50)	41.7	86.25	60.8 (24.66)	45.3	9.79
300	71.7 (10.10)	96.7 (3.82)	72.9	28.68	95.0 (2.50)	74.5	22.11

[a] Standard deviation of three trial means.

To determine whether lipid absorption or abrasion figured more prominently in the interactions of DE and *B. bassiana*, assays were preformed with fungus in combinations with amorphous silicone dioxide and diamond dust (Table 3). There was significant synergistic interaction with both of the desiccants at the higher fungus doses, but only at the higher two doses with the abrasive, diamond dust, as opposed to the higher three doses with the absorbent, silica. Silica also gave larger chi-square values for interaction at all doses. The stronger interaction with silica than with diamond dust suggests that lipid removal contributes more to the observed DE–*B. bassiana* synergism than does abrasion.

Other than hydrocarbons, there is little information on the lipid composition of the insects used in the above experiments. Howard (1992) profiled the cuticular hydrocarbons of sawtoothed grain beetle larvae, Howard and Liang (1993) characterized the cuticular hydrocarbons of larval lesser grain borers, and Howard et al. (1995) profiled the cuticular hydrocarbons of sawtoothed grain beetles through their development. Homologous bands of the major lipid classes were present on thin-layer chromatography plates that had been loaded with cuticular extracts from adult lesser grain borers or with extracts from DE that had been removed from adult lesser grain borers (unpublished data). More extensive profiling of lipids and their uptake by DE will help to elucidate the nature of both the mode of action of DE and how it interacts with entomogenous fungi.

Conidia Adhesion. Treatment with DE was associated with a modest, nonsignificant increase in the number of spores attached to the abdominal cuticles of adult lesser grain borers. The means were 124.4 conidia/mm² (95% CL = 109.0, 139.4) for the diatomaceous earth-treated beetle and 105.6 conidia/mm² (95% CL = 91.5, 119.7) for beetles treated with *B. bassiana* alone. The means are not significantly different ($t = 1.81$, df = 79, $P = .075$), and it is not likely that differential conidia attachment is a major factor in DE-enhanced fungus performance. Germinated conidia were rarely observed on beetle abdomens or other surveyed parts. It appears that the infection process of *B. bassiana* on the tested beetles is very inefficient. In spite of the inefficiency, mortalities in excess of 90% were obtained. This suggests that even a modest increase in infection efficiency may result in a large increase in insect control efficacy.

Effect of Desiccant on Conidia Stability. Like insects, fungus spores are coated with lipids including fatty acids and hydrocarbons (Fisher et al. 1972). Accordingly, their stability in the presence of lipophilic dust is necessary information if the materials are to be used together. When DE and *B. bassiana* spores were incubated in a mix for 75 d at 26 or 30°C, there was no loss of germination rate compared with pure spores. The germination rates of conidia held at 26°C were 82.3% (±SD 1.15) with DE and 80.3% (±SD 8.02) without DE. The germination rates of conidia held at 30°C were 63.3% (±SD 4.04) with DE and 64.7% (±SD 1.15) without DE.

The effects described herein may or may not be large enough to be commercially useful in insect control. If not, the use of desiccant dusts and entomogenous fungi together still makes sense in that they are complementary in their environmental optima. Furthermore, the information developed here can provide a basis for formulating mycoinsecticides with lipophilic materials and, perhaps improve efficacy against a variety of target insects. The effects of the combination described here should be determined for both adults and immature stages of other stored-product insect pests.

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